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BRIEF COMMUNICATION

Genetic Factors in Conditioned Tolerance to the Analgesic Effects of Etonitazene

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ELMER, G. I., C. B. MATHURA AND S. R. GOLDBERG. *Genetic factors in conditioned tolerance to the analgesic effects of etonitazene.* PHARMACOL BIOCHEM BEHAV 45(1) 251–253, 1993.—Tolerance to the analgesic effects of opioids have been reported to include unconditioned (physiological) and conditioned (associative) components. The purpose of the current study was to investigate the genetic and environmental factors important in the development of tolerance by using three inbred strains of mice with varying opiate receptor concentration and acute behavioral response to opioids: C57BL/6J, CXBK/ByJ, and CXBH/ByJ mice. Each strain was divided into three groups; each group received two injections per day as part of the tolerance development procedure. One group of each strain was administered an opioid (etonitazene) specifically paired with the testing room and a second injection of saline in the home colony (paired). The second group of each strain was administered saline in the testing room and etonitazene in the home colony (unpaired). A third group of each strain was administered saline in both the testing room and home colony (control). Etonitazene and saline were administered in this manner for 4 days. On day 5, animals were tested for hot-plate analgesic response following administration of etonitazene. There was a significant effect of treatment condition (paired, unpaired, control) on etonitazene-induced analgesia. In all strains, only the paired treatment condition demonstrated tolerance to the analgesic effects of etonitazene compared to the control groups. There was no significant effect of genotype or a genotype × condition interaction. Thus, genotype significantly affects the acute analgesic effects of opioids but did not affect the development of conditioned tolerance. Consideration of both components of the drug response, genetic and environmental, will lead to a better understanding of factors associated with the development of tolerance and the importance of environmental stimuli.

Genetics Analgesia Tolerance Conditioning Etonitazene CXBK/ByJ Mice

REPEATED administration of an opioid leads to a decrease in its analgesic effects. The development of tolerance can be acquired through unconditioned means (physiological) and through conditioned means (associative). At the unconditioned level, tolerance to an opioid's effects may be related to the physiological or neuronal adaptation to chronic drug exposure such as a functional uncoupling of opiate receptors from their G proteins and an eventual decrease in opiate receptor concentration (12). At the conditioning level, tolerance to an opioid's effects may be related to learning processes associated with the presentation of stimuli previously paired with drug administration (8,10). The two means of tolerance acquisition can be dissociated to some degree by manipulation of the dose and interdose interval (11). However, it is not known what the relative importance of unconditioned and conditioning effects are in an individual's acquisition or expression of tolerance.

Dissociation of a drug response into biological (unconditioned) and environmental (conditioned) components can be done using a behavior genetics approach. When a number of inbred strains are tested in the same environment, variability across strains in response to the drug is due to genetic or biological factors while variability within each strain is due to environmental factors. Similar to environmental factors, genetic factors have been demonstrated to contribute significantly to the acute analgesic properties of opioids (2) and to the degree of tolerance exhibited following repeated drug administration (6). The observed difference in the degree of tolerance across genotype is usually presumed to be related to neuronal mechanisms associated with acute tolerance (3,6,7).

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However, individual differences in the development of tolerance may be related to individual differences in conditioning factors and unrelated to the biological mechanisms involved in acute analgesia.

The purpose of the present study was to determine if genotype and environment both play an important role in the expression of tolerance under conditions known to have a strong environmental impact. Three inbred strains, C57BL/6J, CXBH/ByJ, and CXBK/ByJ, were chosen based upon previous studies that demonstrated significant differences in opiate receptor concentration, opioid-induced analgesia (1,3,4) and naloxone-precipitated withdrawal (7). The use of inbred strains that vary significantly in opiate receptor concentration and behavioral response to opioids help determine the relative contribution of environmental and genetic factors important in the acquisition of tolerance to the analgesic effects of opioids.

METHOD

Animals

Adult male C57BL/6J (C57), CXBK/ByJ (BK), and CXBH/ByJ (BH) mice (Jackson Laboratories and Addiction Research Center), 6–9 months old and weighing approximately 21–26 g at the start of the experiment, were used. Male and female BH and BK mice bred at the Addiction Research Center consisted of first- and second-generation offspring from Jackson Laboratory parental strains. All animals were experimentally naive, housed in groups of three to five in a temperature-controlled room (26°C) with a 12 L : 12 D cycle (light 0700–1900), and given free access to Purina Laboratory Chow and tapwater during the entire experimental procedure.

Procedure

Subjects were randomly divided into three groups: paired, unpaired, and controls. These designations were balanced within cages.

All subjects received two injections per day. Subjects in the paired group received etonitazene in the test room and saline in the colony room. Subjects in the unpaired group received saline in the test room and etonitazene in the colony room. Thus, subjects in the paired and unpaired groups received the same total amount of drug per day; only the location of the drug injection differed between groups. Subjects in the control group received saline injections in the test room and in the colony room.

To determine differences in the development of tolerance across genotype, it is important that the dose of the opioid, etonitazene, produce an equivalent pharmacological effect when given acutely. Otherwise, tolerance to the chronic administration of the opioid would not develop from equivalent baseline levels. Therefore, to compensate for significant differences across strain in sensitivity to acute opioid-induced analgesia the dose of etonitazene required to produce an equal analgesic effect in each strain was determined in pilot studies (data not shown). The dose of etonitazene used in each strain produced an increase of approximately 300% in paw-lick latency as compared to each strain's respective saline-treated control. The training dose of etonitazene for each strain was as follows; 34.8, 30.0, and 10.3 μg/kg for the BK, C57, and BH mice, respectively. Etonitazene was administered SC in a volume of 0.01 ml/g body weight.

Subjects received two injections per day, at approximately 1000 and 1500 h, for 4 days. In the morning session, animals in the paired group received etonitazene injections in the test room; the unpaired and control group received saline in this room. The dimensions of the test room were 2.5 × 3.0 m. The houselight was on continuously during the exposure period. Thirty minutes after their respective injections, all subjects were given two 30-s exposures to the hot-plate apparatus (Socrel DS37), which was maintained at room temperature. All subjects remained in the test room for 3 h in plastic cages without bedding. Approximately 5 h following the morning injections, subjects were transported to the colony room for the afternoon injections. Subjects in the paired and control groups were administered saline, while subjects in the unpaired group were administered etonitazene. All subjects were then placed in their home cages.

On day 5, all animals were administered etonitazene in the morning session. The dose of etonitazene used during training for each strain was administered 30 min prior to the analgesic test conducted on the hot-plate apparatus, which was now maintained at 55°C. Latency to paw-lick was the dependent measure. If the subject failed to paw-lick after 40 s, the test was terminated to avoid tissue damage.

RESULTS

Figure 1 illustrates the effect of environmental stimuli on the development of tolerance to etonitazene-induced analgesia in BH, C57, and BK mice. The doses of etonitazene chosen for each strain were equally effective across strain, that is, there was no significant difference in the percent of maximal analgesia achieved as a function of genotype under control conditions: paw-lick latency, $F(2, 23) = 1.76$, n.s. Therefore, the values in Fig. 1 are presented as a percent of each strains control group value. There was a significant effect of condition on etonitazene-induced analgesia: condition, $F(2, 58) = 1.76$, $p < 0.003$. Compared to the control group, which received saline in both the experimental room and colony, the paired group was the only condition to demonstrate tolerance to the analgesic effect of etonitazene. The unpaired group was

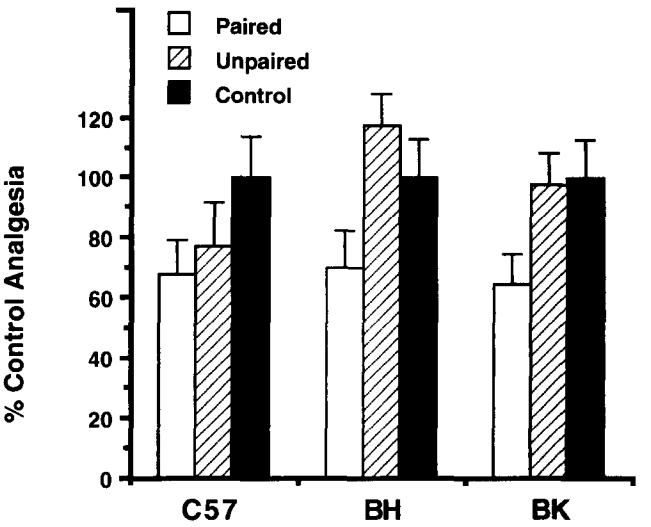

FIG. 1. Tolerance to the analgesic effects of etonitazene as a function of genotype and environmental conditions. Each strain was given a dose of etonitazene that produced an equal acute analgesic effect: 34.8, 30.0, and 10.3 μg/kg for the BK, C57, and BH mice, respectively. Each bar represents the mean (± SEM) of six to nine animals.

not significantly different than the control group. There was no significant effect of genotype on the development of tolerance or a significant genotype × condition interaction. The analgesic effects of etonitazene in the paired group were 68, 68, and 65% of control for BH, C57, and BK mice, respectively. The BH unpaired group was significantly different than the paired group (Duncan's new multiple-range test, $p < 0.05$).

DISCUSSION

Despite large genetic differences in acute sensitivity to etonitazene-induced analgesia and large differences in μ-receptor concentration, genotype did not significantly affect the development of conditioned tolerance. Regardless of genotype, subjects given repeated injections of an equianalgesic dose of etonitazene paired with the test environment stimuli showed significantly greater tolerance to etonitazene-induced analgesia. Thus, acute sensitivity and innate opiate receptor concentration were not related to the development of conditioned tolerance under these training procedures.

An individual's response to acute or chronic administration of a drug is a function of the individual's genotype, the present environmental context, and drug history. However, few studies have investigated genotype × environment interactions associated with drugs of abuse. These data indicate a significant influence of environmental factors on development of tolerance, in particular the effects of stimuli paired with the acute pharmacological response to opioids. The present results serve to emphasize the importance of environmental factors in response to chronic drug administration.

The effect of genotype on the development of tolerance is an important component in phenotype expression. The results of the present study do not suggest an overriding effect of environment because genotype is an important component of the acute response. However, if relative differences in innate sensitivity to opioids are accounted for genotype does not appear to significantly affect learned tolerance under these conditions. Future studies will investigate the context-dependent nature and time course of tolerance development as a function of genotype. Consideration of both components of the drug response, the biological response to the drug and the learning processes associated with the environmental conditions, will lead to a better understanding of factors associated with the development of tolerance.

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REFERENCES

1. Baran, A.; Shuster, L.; Eleftheriou, B. E.; Bailey, D. W. Opiate receptors in mice: Genetic differences. Life Sci. 17:633–640; 1975.
2. Belknap, J. K.; O'Toole, L. A. Studies of genetic differences in response to opioid drugs. In: Harris, R. A.; Crabbe, J. C., eds. The genetic basis of alcohol and drug actions. New York: Plenum Press; 1992:225–252.
3. Gwynn, G. J.; Domino, E. Genotype-dependent behavioral sensitivity to mu vs. kappa opiate agonists. II. Antinociceptive tolerance and physical dependence. J. Pharmacol. Exp. Ther. 231: 312–316; 1984.
4. Moskowitz, A. S.; Goodman, R. R. Autoradiographic analysis of mu1, mu2, and delta opioid binding in the central nervous system of C57BL/By and CXBK (opioid receptor-deficient) mice. Brain Res. 360:108–116;1985.
5. Moskowitz, A. S.; Terman, G. W.; Carter, K. R.; Morgan, J. M.; Liebeskind, J. C. Analgesic, locomotor and lethal effects of morphine in the mouse: Strain comparisons. Brain Res. 361:46–51; 1985.
6. Oliverio, A.; Castellano, C. Genotype-dependent sensitivity and tolerance to morphine and heroin: Dissociation between opiate-induced running and analgesia in the mouse. Psychopharmacologia 39:13–22;1974.
7. Oliverio, A.; Castellano, C.; Eleftheriou, B. E. Morphine sensitivity and tolerance: A genetic investigation in the mouse. Psychopharmacologia 42:219–224; 1975.
8. Seigel, S. Evidence from rats that morphine tolerance is a learned response. J. Comp. Physiol. Psychol. 89:498–506; 1975.
9. Suzuki, T.; Hayashi, Y.; Misawa, M. The role of μ1 receptor in physical dependence on morphine using the μ receptor deficient CXBK mouse. Life Sci. 50:849–856; 1992.
10. Tiffany, S. T.; Maude-Griffin, P. M. Tolerance to morphine in the rat:Associative and nonassociative effects. Behav. Neurosci. 102:534–543; 1988.
11. Tiffany, S. T.; Maude-Griffin, P. M.; Drobes, D. J. Effects of interdose interval on the development of associative tolerance to morphine in the rat: A dose-response analysis. Behav. Neurosci. 105:49–61; 1991.
12. Werling, L. L.; McMahon, P. N.; Cox, B. M. Selective changes in mu opioid receptor properties induced by chronic morphine exposure. Proc. Natl. Acad. Sci. USA 86:6393; 1989.